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Diagnosis of Trisomy 18 by Examining Fetal Karyotype in High-Risk Cases of the First Trimester of Screening Test

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ABSTRACT

Early detection of trisomy 18 during pregnancy and its termination can prevent disabled infants'birth who are a burden on the family and society. The aim of this study was to identify high-risk individuals by performing screening tests in the first trimester of pregnancy and to identify these cases by examining the karyotype. The present prospective study was conducted on 2421 pregnant women with agegestation between 11+0 and 13+6 weeks who were referred to Narges Genetics Laboratory in Ahvaz from 2016 to 2017. This screening was done using a combination of maternal age and fetal nuchal translucency (NT) and crown-rump length (CRL), biochemical markers serum free β-human chorionic gonadotrophin (free β-hCG) and pregnancy associated plasma protein A (PAPP-A). Thereafter, Regression and Chi-square test wereperformed to analyze the obtained data. According to the results of screening tests in the first trimester of pregnancy, 160 cases were included in the high-risk group. After performing karyotype examinations, it was found that 15 cases had trisomy 18. Afterward, in patients with trisomy 18, the mean NT was calculated as 4 mm and the mean CRL was 68 mm, the multiples of the median (MoM) was of PAPP-A 0.2 mg l^{-1}, and free β-hCG was 0.3 ng ml^{-1}. The results of the present study demonstratethat by performing screening tests in the first trimester, considering the age of the mother and gestational age, the baseline risk for fetal anomalies can be detected and using specific software, congenital anomalies can also bediagnosed in the first trimester of pregnancy. And prevented the birth of a baby with a congenital anomaly.

Keywords: Trisomy18, First pregnancy trimester, Nuchal translucency (NT), Chorionic gonadotropin

INTRODUCTION

Trisomy 18, known as Edward syndrome, is a common chromosomal disorder among pregnant women, which is characterized by the presence of an extra chromosome on chromosome 18 [1]. The cause of this abnormality is due to the non-segregation of chromosomes at the time of meiosis division, in which 50% of the segregation occurs in meiosis II maternal Oogenesis [2]. This anomaly was firstly introduced by Edwardin 1960 [3]. Trisomy 18 is known as the second congenital anomaly after Down syndrome. The prevalence of trisomy 18 in newborns is between 1/6000 and 1/8000, but in general, due to the abortion of a large number of fetuses or prenatal diagnosis and termination of pregnancy, its prevalence has been reported to be 3600.1 cases [4,5]. Aninfant with Edward's syndrome (trisomy 18) usually has some characteristics such as mental retardation, congenital heart defect, drooping corners, flexion of the fingers, kidney disorders, syndactyly and skeletal system abnormalities [6]. Since congenital anomalies like trisomy 18 have economic, social, and cultural consequences for families and society, early detection of anomalies in the first trimester of pregnancy and the subsequent termination of such pregnancies can prevent these complications [7]. Notably, the only way to prevent the birth of infants with congenital anomalies is prenatal diagnosis [8]. In the screening test of the first trimester of pregnancy, to estimate the risk of anomaly, the measurement of the thickness of the

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back of the NT fetal neck using ultrasound is an effective way to diagnose trisomy 18. In this regard, the increased NT is associated with an increased incidence of chromosomal abnormalities [9,10]. Ultrasound also shows abnormal placement of the hands in 20% of cases as well as heart defects in 6% of cases [11]. In screening in the first trimester of pregnancy, maternal serum biomarkers including free placental gonadotropin free βhCG and maternal blood plasma protein PAPP_A as well as maternal ageare calculated [12]. Correspondingly, in explaining the results, low levels of multiples of the median (MoM) PAPP-A in maternal serum can be an important indicator for the following chromosomal abnormalities, including trisomy 18 [13]. Performing screening tests in the first trimester could help to identify high-risk individuals [14]. Thereafter, chromosomal abnormalities can be diagnosed with high accuracy by cytogenetic methods and using some samples such as amniotic fluid, chorionic villus sampling (CVS), and cell-free DNA (cfDNA) tests [15,16].

MATERIALS AND METHODS

This prospective study was performed on 2421 pregnant women with gestational age between 11 and 13 weeks and 6 days who were referred to the Narges Laboratory in Ahvaz from 2016 to 2017. At first, the study conditions were fully explained to all pregnant women. The inclusion criteria included single fetus and pregnancy in the first trimester of pregnancy, no maternal disease, and no specific medication consumption. All the included pregnant women were then examined by a skilled ultrasound specialist who was qualified to perform this type of ultrasound by the Scientific Society of Radiology and also had the approval of the Fetal Medicine Foundation (FMF) in the Narges Laboratory in Ahvaz. Afterward, crown-rump length (CRL) and folds of the neck of the Nuchal Translucency (NT) fetuses were measured and recorded in a questionnaire. Subsequently, blood samples were taken from pregnant women and MOMPAPP-A and MOMfβhCG were then evaluated by a cryptographic device. In this questionnaire, mother's age, mother's weight, number of deliveries, multiple births, history of specific disease, family history of genetic disease, family relationship between parents, history of giving birth to a baby with congenital anomaly, and smoking at the time of performing screening and genetic tests were recorded. Moreover, if there was any history of medical condition including diabetes, hypertension or disease of other organs or any adverse pregnancy history including preeclampsia, gestational diabetes, intrauterine death, intrauterine growth restriction,low birth weight, birth defects with congenital anomalies, twin or multiple pregnancies in previous pregnancies, and abortions, pregnant women were excluded from the study. All information obtained from the participants in the first trimester of pregnancy were given to the software (FMF). After this stage, 160 pregnant women were included in the high-risk group. To diagnose trisomy 18, karyotype examinations were performed on all the subjects in the Narges Laboratory.

The obtained data were analyzed using SPSS18 software. Data analysis was performed using Regression and Chi-square tests. The p value less than 0.05 was considered as statistically significant. In addition, High-risk individuals were examined for their karyotypes, and low- and moderate-risk populations were then followed for abnormalities in their children.

RESULTS

In this research, 2421 pregnant women with gestational age between 11 to 13 weeks and 6 days in the first trimester of pregnancy were studied. 2033 people were enrolled in the low risk group (84%), 228 people in the medium risk group (9.4%), and 160 (6.6%) were included in the high risk group. The karyotype of all high risk people was examined. Afterward, it was found that 15 cases were with trisomy 18 and 145 fetuses were normal .Among them, the number of people with normal karyotype was 65 (40.6%) under 35 years old and 80 (50.1%) over 35 years old. The number of people with trisomy 18 under 35 years old was 7 people (4.3%) and the number of people with this disorder over 35 years old was 8 people (5%) (Fig. 1).

The examination of the subjects' personal and family information showed that 14 pregnant women had a history of anomalies in their previous children. Accordingly, these anomalies included Down syndrome (n = 6), mental retardation (n = 4), trisomy 18, amphalocele, deaf and hydrocephalus (n = 1). The mean age of women included in

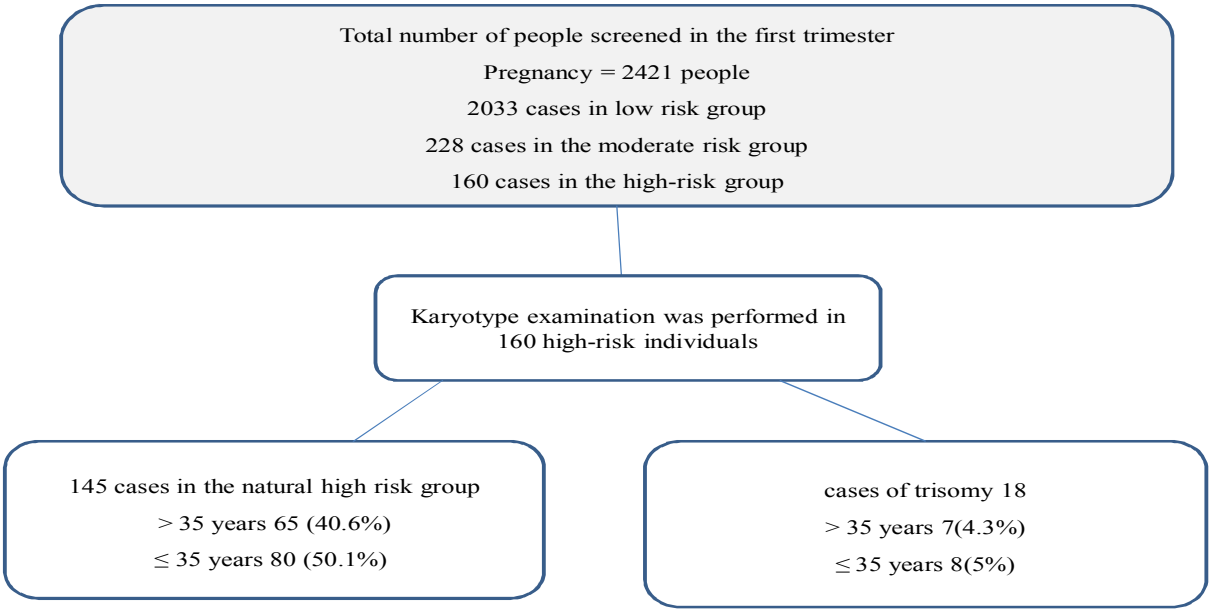

Fig. 1. Characteristics of the subjects.

Table 1. Age, NT and CRL

	Age	NT	CRL
Low risk	25	1.7	56.8
	(20-38)	(1-2.2)	(54.1-82)
Medium risk	26	1.9	58.7
	(21-40)	(1.3-3.2)	(56.9-85)
High risk	33	3.8	66.7
	(25-43)	(2.9-5.3)	(58.5-80.5)
<35 years	29	4	66.6
	(25-34)	(2.9-5.1)	(58.5-80.5)
≥35 years	37	3.9	66.8
	(35-43)	(2.8-5.3)	(59-80.1)

the low-risk group was 25 years old (20-38), the mean age of women in the medium-risk group was 26 years old (21-40), and the mean age of women the high-risk group was 33 years old (25-43) (as stated, the mean age of people in the high-risk group was under 35 years old). Year 29 years (34-25) and people over 35 years 37 years (35-43). The

mean weight of women BMI was 73.5 ± 13.2 (42-120) kg. Moreover, the mean NT in the low risk group was 1.7 (1-2.2) mm, in the medium risk group it was 1.9 (1.3-3.2) mm, and in the high risk group it was 3.8 mm (2.9-5.3). In addition, in women under 35 years old it was 4 (2.9-5.1) mm, and in women over 35 years old it was 3.9 (2.8-5.3) mm. Average CRL in low risk group was 56.8 (54.1-82) mm and in the moderate risk group it was 58.7 (56.9-85) mm, and in the high risk group it was 66.7 mm (58.5-80.5). Additionally, in women under 35 years old, it was 66.6 (58.5-80.5) mm, and in women over 35 years old, it was 66.8 (59-80.1) (Table 1).

The mean PAPP_A mg l^{-1}) in the low risk group was 1.2 (1-1.5), in the medium risk group was 0.9 (0.5-2.8), and in the high risk group was 0.3 (0.2-1.6). Furthermore, it was 0.3 (0.2-1.6) in women under 35 years old and 0.4 (0.2-1.8) in women over 35 years old. The mean fβhCG ng ml^{-1}) in the low risk group was 1 (0.9-1.6), in the medium risk group was 1 (0.8-4), and in the high risk group was 0.4 (0.2-4). Moreover, in women under 35 years old, it was 0.6 (0.7-4) and in women over 35 years old, it was 0.6 (0.4-2) (Table 2).

After karyotype examinations, it was found that, out of 160 high-risk individuals, 15 cases had 18 trisomy. The mean age of women with trisomy 18 fetuses was 33 years old (20-40), which was 29 years old (20-31) for women under 35 years old and 38 (35-40) for women over 35 years old. Mean NT 4 (3.8-6.3) mm in women under 35 years old was 3.9 (3.6-5.5) mm and in women over 35 years old, it was 4.5 (3.8-6.3) millimeters. The average CRL was 68 mm (61.2-80.5) mm, which was 66.9 (61.2-70.1) in women under 35 years old and 67.6 (67-80.5) in women over 35 years old. Figure 2 shows a comparison of the mean NT and CRL in the low, medium and high risk groups as well as Persons with trisomy 18.

The mean PAPP_A was 0.2 (0.1-0.3) mg l^{-1}. Accordingly, in women under 35 years old it was 0.3 (0.2-0.3) and in women over 35 years old was 0.1 (0.1-0.3). The mean fβhCG 0.3 (0.3-0.4) ng ml^{-1} in women under 35 years old was 0.3 (0.2-0.3) and in women over 35 years old was 0.3 (0.3-0.4) (Table 3). Figure 3 shows a Comparison of the mean PAPP_A and fβhCG the low and medium risk groups and Persons with trisomy 18.

Based on the above mentioned estimations, the prevalence rate of trisomy 18 was 5% per 1000 patients. (Table 3). The relationship among NT, CRL, PAPP-A and fβhCG screening tests in the first trimester of pregnancy was assessed by Chi-square test, which was found to be statistically significant. Also, the P value less than 0.05 was considered as significant. In this study, the positive rate of Kadab was 5.9 and the sensitivity was 100. The karyotype image of a fetus with trisomy 18 obtained from our results in the lab was presented in Fig. 4.

DISCUSSION

Various studies showed that by performing screening in the first trimester of pregnancy and evaluating the NT and serum of maternal blood markers including PAPP-A and fβhCG, diagnosis of trisomy 18 abnormality can be made [17]. The results of the present study show that high-risk cases and trisomy 18 can be identified by screening in the first trimester of pregnancy. In a study by Tang *et al.* (2019), NT and serum marker PAPP_A were used to identify trisomy 18 in the first trimester of pregnancy [18]. In a study by Dugoff *et al.* (2007), it was reported that there was no significant relationship between theincreased fetal NT and chromosomal abnormalities [19]. Accordingly, this study result was not consistent with that of the present study. In another study, Lee *et al.* (2017) screened participants for trisomy 18 in the first trimester of pregnancy. Fetal NT levels as well as serum of maternal blood markers including PAPP_A and fβhCG, were also evaluated. In this study, after karyotype examinations, it was found that the amount of NT is much higher in people with trisomy 18 compared to normal people. Moreover, in patients with trisomy 18, PAPP_A levels were found to be associated with a decrease in their levels compared to normal individuals [20]. Correspondingly, this was consistent with that of the present study. In a study by Toring *et al.* (2016), there was a significant relationship between PAPP_A level and the incidence of chromosomal abnormalities, including trisomy 18 [21]. Moreover, ina study by Engel *et al.* (2017) and after analyzing maternal blood marker serum with cryptor, a decrease was observed in PAPP_A and fβhCG levels in patients with trisomy 18 [22]. In a study by Nichols *et al.* (2011), after ultrasound examination in the first trimester of pregnancy, it was stated

Table 2. Mean PAPP_A and fβhCG

	PAPP_A	fβhCG
Low risk	1.2	1
	(1-1.5)	(0.9-1.6)
Medium risk	0.9	1
	(0.5-2.8)	(0.8-4)
High risk	0.3	0.4
	(0.2-1.6)	(0.2-4)
<35 years	0.3	0.6
	(0.2-1.6)	(0.7-4)
≥35 years	0.4	0.6
	(0.2-1.8)	(0.4-2)

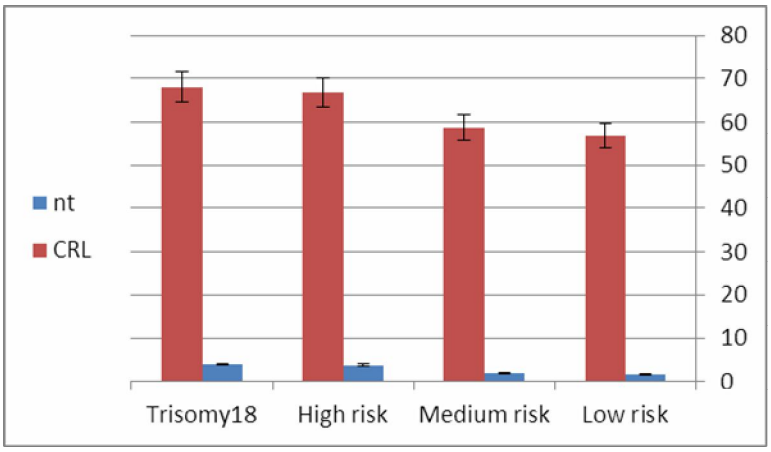

Fig. 2. Compare the mean NT and CRL in each of the four groups.

that there is a significant relationship between the increased fetal NT and trisomy 18 [23]. In a study by Bestwick *et al.* (2013), it was reported that people with trisomy 18 had an NT above 2.5 mm, so they were included in the high-risk group [24]. In a study by Shifa *et al.* (2013), the mean NT of patients with trisomy 18 was 3.2 mm, MOMPAPP_A was 0.1, and the mean fβhCG was 0.2 MOM [25]. Accordingly, this was consistent with the result of the present study. Spencer *et al.* (2005) in their study stated that there was no significant relationship among the first trimester screening, NT measurement, PAPP_A and fβhCG levels, and the incidence of trisomy 18 [26],which was not consistent with the result of the present study. Additionally, in a study by Carrara *et al.* (2019), after screening the first trimester of pregnancy and performing NT ultrasound tests and serum markers of PAPP_A and fβhCG markers, high-risk individuals were identified and 18 karyotype tests were then performed to diagnose trisomy [27]. First trimester

Table 3. Mean Age, NT, CRL, PAPP-A and fβhCG in Patients with Trisomy 18

	Age	NT	CRL	PAPP_A	fβhCG
With trisomy 18	33	4	68	0.2	0.3
	(20-40)	(3.8-6.3)	(61.2-80.5)	(0.1-0.3)	(0.3-0.4)
<35 years	29	3.9	66.9	0.3	0.3
	(20-31)	(3.6-5.5)	(61.2-70.1)	(0.2-0.3)	(0.2-0.3)
≥35 years	38	4.5	67.6	0.1	0.3
	(35-40)	(3.8-6.3)	(67-80.5)	(0.1-0.3)	(0.3-0.4)

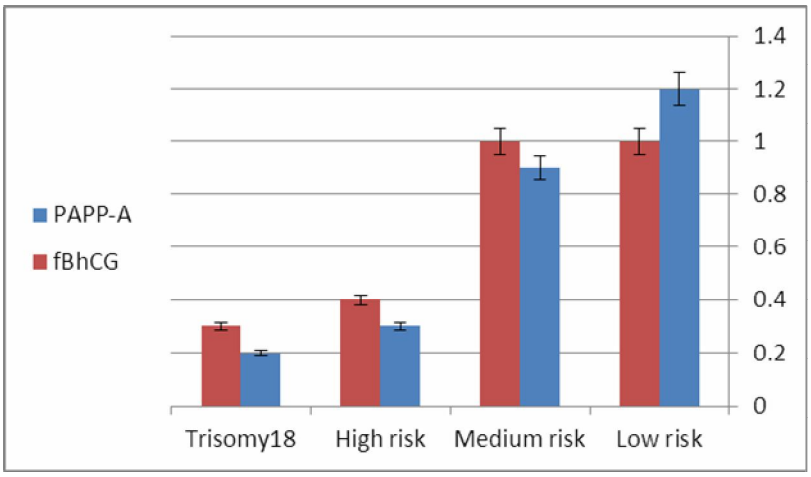

Fig. 3. Compare the mean PAPP-A and fβhCG in each of the four groups.

screening has been used in the country for many years to diagnose congenital anomalies. But the karyotype of the fetus has been examined by method CfDNA for the first time in the country in the Narges Genetics Laboratory of Ahvaz.

CONCLUSIONS

The results of the present study demonstrate that by performing screening tests in the first trimester, considering the age of the mother and gestational age, the baseline risk for fetal anomalies can be detected and using specific software, congenital anomalies can also be diagnosed in the first trimester of pregnancy. And prevented the birth of a baby with a congenital anomaly. The advantage of screening in the first trimester of pregnancy is the early detection of congenital anomalies to prevent the birth of a child with a congenital anomaly and to perform a medical abortion before the fetus grows further.

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Fig. 4. Karyotype image of a fetus with trisomy 18.

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Declarations

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Conflicts of interest/Competing interests

The authors declare no competing interests.

Availability of Data and Material

Supplemental data are provided.

Authors' Contributions

All authors contributed to the study conception and design. The biological experiments and data collection were done by Mozhgan Harfsheno and Akram Roohandeh. The first draft of the manuscript was written by Garshasb Rigi and Mozhgan Harfsheno and all authors commented on previous versions of the manuscript. Mojgan Harfsheno as a corresponding author and Mozhgan Barati as an adviser of the study, designed the whole experiments and supported the project. Garshasb Rigi analyzed data and wrote the final manuscript. All authors read and approved the final manuscript.

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